PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, 5/10, C07K 14/705, G01N 33/50, 33/60	A1	(11) International Publication Number: WO 95/23858 (43) International Publication Date: 8 September 1995 (08.09.95)

(21) International Application Number: PCT/US95/02221

(22) International Filing Date: 23 February 1995 (23.02.95)

(30) Priority Data:

08/207,401	4 March 1994 (04.03.94)	US
08/288,405	10 August 1994 (10.08.94)	US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US).

(72) Inventors: CHANDY, Kanianthara, George; 1218 Morningside Drive, Laguna Beach, CA 92651 (US). KALMAN, Katalin; 2017B Los Trancos, Irvine, CA 92715 (US). CHANDY, Grischa; 3122 Verano Place, Irvine, CA 92715 (US). GUTMAN, George, A.; 16350 Harbor Boulevard #2208, Costa Mesa, CA 92626 (US).

(74) Agent: BERLINER, Robert; Robbins, Berliner & Carson, 5th floor, 201 N. Figueroa Street, Los Angeles, CA 90012-2628 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

With international search report.

(54) Title: A NOVEL VOLTAGE-GATED POTASSIUM CHANNEL GENE

(57) Abstract

This disclosure relates to the identification of a new voltage-gated potassium channel gene, Kv1.7, which is expressed in pancreatic β-cells. The invention utilizes this new potassium channel for assays designed to identify extrinsic materials with the ability to modulate said channel for the development of therapeutics effective in the treatment of non-insulin-dependent diabetes mellitus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

A NOVEL VOLTAGE-GATED POTASSIUM CHANNEL GENE

Cross-Reference to Related Applications

This is a continuation-in-part of U.S. application Serial No. 08/207,431, filed March 4, 1994.

Reference is hereby made to the following related applications: Serial No. 07/955,916, filed October 2, 1992 and Serial No. 08/170,418, filed December 20, 1993, and to their parent applications, all of which being hereby expressly incorporated by reference.

Field of the Invention

The present invention relates to the identification of a new voltage-gated potassium channel gene, Kv1.7, which is expressed in the rat and hamster insulinoma cell lines, RINm5F and HIT, respectively. Since voltage-gated potassium channels modulate insulin secretion from pancreatic β-cells, selective Kv1.7 blockers would be expected to increase insulin release and thereby reduce hyperglycemia associated with non-insulin-dependent diabetes mellitus.

The present invention is also directed toward assays for testing extrinsic materials for their ability to block the Kv1.7 channel, and thereby exert an effect on insulin secretion from β-cells. To this end, we have generated an expression construct, containing the coding region of the Kv1.7 gene and have demonstrated that this gene, when expressed in *Xenopus* oocytes, encodes a voltage-dependent, rapidly-activating, non-inactivating delayed rectifier-type channel that is both tetraethylammonium- and 4-aminopyridine-resistant. This construct can now be used for the development of mammalian cell lines expressing this channel; such cell lines could be used in high-throughput screening assays of extrinsic materials.

Background of the Invention

Mammalian cell membranes perform very important functions relating to the structural integrity and activity of various cells and tissues. Of particular interest in membrane physiology is the study of trans-membrane ion channels which act to directly control a variety of physiological, pharmacological and cellular processes. Numerous ion channels have been identified including calcium (Ca), sodium (Na) and potassium (K) channels, each of which have been analyzed in detail to determine their roles in physiological processes in vertebrate and insect cells.

A great deal of attention has recently been focused on the potassium channel because of its involvement in maintaining normal cellular homeostasis. A number of these potassium channels open in response to changes in the cell membrane potential. Many voltage-gated potassium channels have been identified and are distinguishable based on their electrophysiological and pharmacological properties. An extended family of at least twenty genes have been isolated, each encoding functionally distinct voltage-gated potassium channels, and each with a unique tissue distribution pattern. Several of these have been shown to be involved in maintaining the cell membrane potential and controlling the repolarization of the action potential in neurons, muscle and pancreatic β-cells. Potassium currents have been shown to be more diverse than sodium or calcium currents and also play a role in determining the way a cell responds to an external stimulus. The diversity of potassium channels and their important physiological role highlights their potential as targets for developing therapeutic agents for various diseases.

Type II or non-insulin-dependent diabetes (NIDDM) is a chronic and debilitating disorder affecting at least 5% of the human population (Bell, G.I. et al., 1980, Nature **284**:26 and Horst-Sikorska, W. et al., 1994, Hum. Genet.

93:325). NIDDM, manifested as fasting hyperglycemia, results either from a defect in insulin release from pancreatic β-cells or from the inability of peripheral tissues to respond appropriately to insulin (Bell, G.I. et al., 1980, supra, Horst-Sikorska, W. et al., 1994, supra and Herman, W.H. et al., 1994, Diabetes **43**:40).

Current therapeutic management of this disease is based primarily on the use of drugs (sulfonylurea compounds) that enhance insulin release by selectively modulating K_{ATP} channels (Boyd III, A.E., 1988, Diabetes **37**:847, Rajan, A.S. et al., 1990, Diabetes Care **13**:340, Misler, S. et al., 1986, Proc. Natl. Acad. Sci USA **83**:7119, Petersen, O.H. and Findlay, I., 1987, Physiol. Rev. **67**:1054 and Ashcroft, F.M., 1988, Ann. Rev. Neurosci. **11**:97). Hypoglycemia is a frequent side effect of such anti-diabetic therapy because these drugs, mimicking the action of glucose, induce membrane depolarization of β-cells (Bell, G.I. et al., 1980, supra, Horst-Sikorska, W. et al., 1994, supra and Herman, W.H. et al., 1994, supra, Boyd III, A.E., 1988, supra, Rajan; A.S. et al., 1990, supra, Misler, S. et al., 1986, supra, Petersen, O.H. and Findlay, I., 1987, supra, Ashcroft, F.M., 1988, supra, Dukes, I. et al., 1994, J. Biol. Chem. **269**:10979, Cook, D.L. et al., 1991, Trends Neurosci. **14**:411, Smith, P.A. et al., 1990, J. Gen. Physiol. **95**:1041, Smith, P.A. et al., 1990, FEBS Lett. **261**:187, Atwater, I. et al., 1983, Cell Calcium **4**:451, Ammala, C. et al., 1991, Nature **353**:849 and Worley III, J.F. et al., 1994, J. Biol. Chem. **269**:12359). Sulfonylurea-induced insulin release, therefore, occurs in a glucose-independent manner. A glucose-dependent insulin secretagogue could potentially avoid the debilitating side effect of hypoglycemia, and would therefore be extremely useful.

Another form of treatment in severe long-standing NIDDM is insulin replacement. This approach, although effective, is time-consuming, expensive and requires the

administration of painful injections often many times daily. To say the least, NIDDM patients would welcome a more effective treatment with fewer side effects. An understanding of the mechanisms responsible for insulin secretion may help identify new targets for the development of such novel anti-diabetic drugs.

Transmembrane ion channels are the primary elements that transduce signals in pancreatic β-cells, resulting in the release of insulin (Boyd III, A.E., 1988, supra, Rajan, A.S. et al., 1990, supra, Misler, S. et al., 1986, supra, Petersen, O.H. and Findlay, I., 1987, supra, Ashcroft, F.M., 1988, supra, Dukes, I. et al., 1994, supra, Cook, D.L. et al., 1991, supra, Smith, P.A. et al., 1990, J. Gen. Physiol. **95**:1041, Smith, P.A. et al., 1990, FEBS Lett. **261**:187, Atwater, I. et al., 1983, supra, Ammala, C. et al., 1991, supra and Worley III, J.F. et al., 1994, supra). In response to an elevation in external glucose, the β-cell membrane slowly depolarizes (phase I). This metabolic coupling appears to be due to an increase in cytosolic ATP, which results in the closure of ATP-sensitive potassium (K_{ATP}) channels. The membrane depolarization in turn initiates sinusoidal bursts of calcium action potentials (phase II), during which intracellular calcium rises, triggering insulin secretion (Boyd III, A.E., 1988, supra, Rajan, A.S. et al., 1990, supra, Misler, S. et al., 1986, supra, Petersen, O.H. and Findlay, I., 1987, supra, Ashcroft, F.M., 1988, supra, Dukes, I. et al., 1994, supra, Cook, D.L. et al., 1991, supra, Smith, P.A. et al., 1990, J. Gen. Physiol. **95**:1041, Smith, P.A. et al., 1990, FEBS Lett. **261**:187, Atwater, I. et al., 1983, supra, Ammala, C. et al., 1991, supra and Worley III, J.F. et al., 1994, supra). Voltage-gated potassium channels have been suggested to play a critical role in repolarizing the membrane after each of these calcium spikes.

Alteration in any of these ionic signalling events could interfere with insulin release and result in hyperglycemia. Overexpression of voltage-gated potassium channels, for example, might be expected to excessively hyperpolarize the membrane following each calcium spike and thereby inhibit the reopening of voltage-gated calcium channels with the reduction in calcium entry leading to diminished insulin release and hyperglycemia. We have therefore focused our attention on identifying the pancreatic islet cell voltage-gated potassium channel.

Summary of the Invention

The present invention relates to the identification of a new voltage-gated potassium channel gene, Kv1.7, which is expressed in the rat and hamster insulinoma cell lines, RINm5F and HIT, respectively. Thus, the present invention is predicated on the identification and characterization of a marker molecule in pancreatic β-cells that modulates insulin release and that leads to a general therapeutic target for NIDDM. This predicate, in combination with the generation of an expression construct, makes possible the development of an assay to identify extrinsic materials possessing the ability to selectively modulate the marker and thereby modulate insulin secretion.

Having established a link between potassium channel function and insulin secretion from pancreatic β-cells as a predicate of the present invention, it follows that the present invention is further directed to associated consequential aspects including assays for testing extrinsic materials for their ability to modulate the Kv1.7 potassium channel, and thereby exert an effect on insulin secretion from pancreatic β-cells.

The present invention is further directed to a method for treating NIDDM in an organism manifesting said disease comprising contacting said organism with an

extrinsic material having a modulating effect on Kv1.7 potassium channels, such materials identified by employing the assay system described *supra*.

The present invention is further directed to kits containing the associated structure, reagents and means to conduct screening assays as described *supra*.

Further, the present invention is directed to the foregoing aspects in all their associated embodiments as will be represented as equivalents within the skill of those in the art.

The present invention is thus directed to the management and control of NIDDM including selectively screening for, preferably selective, modulators of Kv1.7 potassium channels for use as a therapeutic.

Brief Description of the Figures

Figure 1A represents the mouse Kv1.7 coding sequence which is indicated by the two stippled boxes. The six bars within these regions indicate the putative membrane-spanning domains S1 through S6. Restriction sites are indicated as follows: BglII (B), EcoRI (E), PstI (P) and SacI (S). The order of restriction sites was determined by single, partial and double digests and by DNA sequencing. Also indicated is a comparison of the genomic sequence of mouse Kv1.7 (SEQ ID NOS:1 and 3) with that of mouse (mKv1.7) (SEQ ID NO:5) and hamster (haKv1.7) (SEQ ID NO:7) cDNAs showing the splice donor and acceptor sites which form the boundaries of the single intervening sequence.

Figure 1B shows the deduced amino acid sequence (SEQ ID NO:10) of mouse Kv1.7. The six putative membrane-spanning domains (S1 through S6) and pore-forming region (P) are also indicated. Potential sites of post-translational modification are shown as follows: N-glycosylation (*); tyrosine kinase (TY-K) and protein kinase C (PKC). Every tenth residue is indicated by a dot above. The hydrophobic core of this protein shares

considerable sequence similarity with other Shaker-family channels, while the intracellular N- and C-termini and the external loops between S1/S2 and S3/S4 show little conservation.

Figure 2 shows Northern blot analysis of total RNA isolated from the hamster insulinoma HIT cell line (H) and rat insulinoma RINm5F cell line (R). The probe used was a PstI/SacI fragment from the Kv1.7-specific 3' untranslated region of the Kv1.7 cDNA. Molecular weight markers are also presented. In both cases a 2.0 kilobase band is observed.

Figures 3A and 3B present the complete nucleotide sequence (SEQ ID NO:9) of the entire coding region for the mouse Kv1.7 gene as compared to portions of the human Kv1.7 gene sequence (SEQ ID NOS:11-19). The mouse Kv1.7 sequence (SEQ ID NO:9) is presented on the top line whereas the bottom line represents the corresponding human Kv1.7 sequence (SEQ ID NOS:11-19). Dashes (-) in the human sequence represent nucleotides that are identical to those presented in the mouse sequence. Open spaces in the human sequence represent regions for which no sequence data is available.

Figure 4 shows the deduced order of two potassium channel genes, hKv1.7 and hKv3.3, on human chromosome 19.

Detailed Description of the Invention

A. Definitions

By the term "extrinsic material" herein is meant any entity that is not ordinarily present or functional with respect to the Kv1.7 potassium channel and/or pancreatic islet cells and that affects the same. Thus, the term has a functional definition and includes known, and particularly, unknown entities that are identified to have a modulating effect on Kv1.7 channel expression, and/or the associated pancreatic islet cells.

By the term "modulating effect", or grammatical equivalents, herein is meant both active and passive impact on the Kv1.7 potassium channel and/or pancreatic islet cells. These include, but shall not be construed as limited to, blocking or activating the channel or the function of the channel protein to materials that ordinarily permeate therethrough, reducing or increasing the number of ion channels per cell and use of secondary cell(s) or channel(s) to impact on a primary abnormal cell.

B. Detailed Description

A new Shaker-related potassium channel gene. We now have identified a novel potassium channel gene, Kv1.7, which belongs to the Shaker-subfamily of genes. A restriction map of a 6.4 kilobase EcoRI DNA fragment containing the entire mouse Kv1.7 coding region is shown in Figure 1A. Unlike all other known mammalian Shaker-related genes (Kv1.1-Kv1.6) that have intronless coding regions (Swanson, R.A. et al., 1990, Neuron **4**:929, Chandy, K.G. et al., 1990, Science **247**:973, Douglass, J. et al., 1990, J. Immunol. **144**:4841, Roberds, S.L. and Tamkun, M.M., 1991, Proc. Natl. Acad. Sci. USA **88**:1798, Tamkun, M.M. et al., 1991, FASEB J. **5**:331, Migeon, M.B. et al., 1992, Epilepsy Res. **6(supp.)**:173 and Shelton, P.A. et al., 1993, Receptors and Ion Channels **1**:25), the protein coding region of mouse Kv1.7 is interrupted by a single 1.9 kilobase intron whose splice sites are shown in Figure 1A. The deduced mouse Kv1.7 protein (SEQ ID NO:10) consists of 532 amino acids and contains six putative membrane-spanning domains, S1-S6 (Figure 1B). The upstream exon encodes the amino terminus and the first transmembrane segment (S1), while the remainder of the coding sequence is contained within the downstream exon.

Expression of Kv1.7 in pancreatic β-cells. Northern blot assays using a Kv1.7-specific 3'-NCR probe revealed a strongly hybridizing 2 kilobase band in the rat and hamster insulinoma lines, RINm5F and HIT (see Figure 2). RINm5F and HIT cells are neoplastic versions of pancreatic β-cells and can secrete insulin in response to glucose challenge like their normal counterparts. These cells have been widely used as models for normal pancreatic β-cells. We have also demonstrated the presence of Kv1.7 mRNAs in these cells by PCR analysis, which we confirmed by sequencing (a portion of the hamster sequence is shown in Figure 1). Betsholtz, C. et al., 1990, FEBS Lett. **263**:121 have also used PCR to amplify a short segment of Kv1.7 cDNA spanning the S5/S6 region from mouse (MK-6), rat (RK-6) and hamster (HaK-6) insulin-producing cells. Our sequence is identical to their MK-6 sequence in the short region of overlap, except for four single nucleotide changes.

These results led us to hypothesize that Kv1.7 is expressed in normal pancreatic islet β-cells, and may play an important role in the electrical events regulating insulin release, making it a potential therapeutic target for NIDDM. To test this idea, we provided Kv1.7-specific DNA probes to Dr. Julie Tseng-Crank at Glaxo, for *in situ* hybridization on histological sections of pancreata from normal and diabetic *db/db* mice. In confirmation of our prediction, Dr. Tseng-Crank found that Kv1.7 mRNA was present in both normal and diabetic islet cells.

Electrophysiological and pharmacological properties of Kv1.7. To study the properties of this channel, we generated an expression construct in which the intron was spliced out, along with the 5'- and 3'-non-coding sequences. This construct, when expressed in *Xenopus* oocytes, encodes a channel which is voltage-dependent, rapidly-activating and non-inactivating, and is TEA- and 4AP-resistant.

Chromosomal location of Kv1.7 in humans. DNA probes from mouse Kv1.7 and Kv3.3 were isolated and sent to the Human Genome (Chromosome 19) Center at Lawrence Livermore laboratory. We had previously demonstrated that Kv1.7 and Kv3.3 were located on human chromosome 19 (Ghanshani, S. et al., 1992, Genomics **12**:190 and McPherson et al., 1991, in Eleventh International Workshop on Human Gene Mapping), and needed more specific localization. Dr. Mohrenweiser's group used these mouse probes to isolate human Kv1.7- and Kv3.3-containing cosmid clones from a chromosome 19 library, and then used the human cosmids as fluorescent-probes for *in situ* hybridization experiments to map both genes to human 19q13.3-13.4. The idiogram of human chromosome 19 shown in Figure 4 indicates that Kv1.7 (KCNA7) is located centromeric of Kv3.3 (KCNC3). Genes for both glycogen synthase (GSY) and the histidine-rich calcium protein (HRC) also map centromeric of Kv3.3, but the order of Kv1.7, HRC and GSY could not be resolved by fluorescence *in situ* hybridization experiments. Studies by S. Elbein and colleagues, however, have placed HRC approximately 4 cM centromeric to GSY.

NIDDM is heterogeneous in its etiology, and families have been described in which the disease is associated with mutations in either glucokinase (chromosome 7) or a gene closely linked to adenosine deaminase (chromosome 20) (Vaxillaire, M. et al., 1994, Diabetes **43**:389, Froguel, P. et al., 1993, N. Eng. J. Med. **328**:697 and Bell, G.I. et al., 1991, Proc. Natl. Acad. Sci. USA **88**:1484). Additional forms of NIDDM exist which are not linked to either of these genes (Vaxillaire, M. et al., 1994, supra, Froguel, P. et al., 1993, supra and Bell, G.I. et al., 1991, supra) and recent studies suggest that a locus predisposing to diabetes exists at human chromosome 19q13.3. First, in a large group of unrelated patients in Finland, a polymorphism of the GSY gene is associated with the development and severity of NIDDM (Groop, L.C. et al., 1993, N. Eng. J. Med. **328**:10 and

Vestergaard, et al., 1993, J. Clin. Invest. **91**:2342). However, there was no evidence for structural defects in the GSY gene or alterations in the total level of GSY protein in these patients, indicating that expression of this gene was unaltered, and suggesting that GSY may only be a marker for another gene on 19q13.3 (Groop, L.C. et al., 1993, supra and Vestergaard, et al., 1993, supra). More recent studies using polymorphic markers in this region exclude the GSY gene as a candidate (Vaxillaire, M. et al., 1994, supra, Froguel, P. et al., 1993, supra, Bell, G.I. et al., 1991, supra, Groop, L.C. et al., 1993, supra and Vestergaard, et al., 1993, supra), and suggest that a diabetic susceptibility gene may lie centromeric to HRC and away from GSY. The localization of the islet cell potassium channel gene, Kv1.7 (KCNA7), to human 19q13.3 and its over-expression in diabetic islets therefore make it a candidate; Kv1.5 was excluded because it is on human chromosome 12p13 (Curren, M. et al., 1992, Genomics **12**:729 and Attali, B. et al., 1993, J. Biol. Chem. **268**:24283), and is not found in islet cells (see above). Thus, Kv1.7 may be a candidate gene for some inherited forms of NIDDM associated with impaired insulin secretion.

Sequence analysis of the human Kv1.7 gene. Numerous partial human Kv1.7 cDNA clones have been isolated using the mouse Kv1.7 cDNA as a probe and sequence data from the human Kv1.7 gene has been obtained. Partial human Kv1.7 sequences (SEQ ID NOS:11-19), in comparison to the sequences of the mouse Kv1.7 coding region (SEQ ID NO:9), is shown in Figure 3. The sequence information in Figure 3 demonstrates that portions of the human Kv1.7 gene possess a great deal of homology with that of the mouse Kv1.7 gene.

Kv1.7-selective blockers could function as glucose-dependent insulin secretagogues. We have shown that Kv1.7 is a novel *Shaker*-related gene encoding a rapidly activating, non-inactivating, TEA-resistant voltage-gated

potassium channel expressed in pancreatic β-cells. Voltage-gated potassium channels with properties similar to Kv1.7 have been reported to regulate membrane repolarization following each calcium spike during phase II of insulin secretion. A Kv1.7 blocker would therefore be expected to lead to glucose-dependent modulation of insulin release, potentially avoiding the debilitating side effect of hypoglycemia. Such drugs would have wide therapeutic use in the management of NIDDM.

Use of the Kv1.7 expression construct to identify Kv1.7-specific glucose-dependent insulin secretagogues. The Kv1.7 expression construct described above has been successfully used to generate functional potassium channels with unique properties. This construct or related ones can be used for expression of functional Kv1.7 channels in mammalian cell lines that do not express endogenous potassium channels (e.g., CV-1, NIH-3T3, or RBL cell lines). These cell lines can then be loaded with ^{86}Rb (Rb ions permeate through potassium channels nearly as well as potassium ions) in the presence of absence of extrinsic materials, and Kv1.7 modifiers identified by their ability to alter ^{86}Rb-efflux. When natural toxins are identified which block Kv1.7 activity, modifiers of Kv1.7 activity could also be identified by their ability to block or reverse the binding of labeled toxins to cells expressing this channel. Compounds discovered in either of these manners could then be formulated and administered as therapeutic agents for the treatment of NIDDM.

C. Materials and Methods

1. Screening of the Mouse Genomic DNA Library

To isolate the Kv1.7 cDNA, approximately 5x10^5 plaques from an AJR/J mouse genomic library were screened (genomic DNA partially digested with the restriction endonuclease Mbo I and cloned into the vector J1, a derivative of L47.1) (a gift of Jonathan Kaye, University

of California, San Diego, La Jolla, California). The genomic library was screened using a mixture of the mouse Kv1.1 (MK1) (Temple et al., Nature **332**:837 (1988)) and rat Kv1.5 (KV1) cDNA (Swanson et al., Nature **332**:837 (1990)) as a probe. Probes were labeled with ^{32}P to a specific activity of $1x10^9$ cpm/ug by the random primer method of Feinberg and Vogelstein, Anal. Biochem. **132**:6 (1983). The mouse Kv1.1 (MK1) cDNA probe containing the entire 1485 base pair coding region was obtained from Bruce Tempel (University of Washington, Seattle, Washington). The 1.1 kilobase fragment probe derived from the rat Kv1.5 (KV1) cDNA, containing the coding region from S3 to its end, was obtained from Leonard Kaczmarek (Yale University, New Haven, Connecticut). Hybridization was performed at 55 °C in hybridization buffer for 16-18 hr. Hybridization buffer consists of 5xSSC, 10x Denhardt's (0.2% bovine serum albumin, 0.2% polyvinyl pyrrolidone), and 0.1% SDS. The blots were washed at a final stringency of 0.5xSSC and 0.1% SDS for 60 min at 55 °C. The blots were then exposed to X-OMAT AR film (Kodak, Rochester, New York) at -70 °C using an intensifying screen.

DNA was isolated from positive phage clones, digested to completion with HindIII and electrophoresed on a 0.9% agarose gel. DNA was transferred to nitrocellulose membranes by capillary transfer and Southern blotting was performed by the method of Southern, Methods in Enzymology (R. Wu, Ed.), **68**:152, Academic Press, New York. Hybridizing and non-hybridizing fragments were then subcloned into the HindIII site of the pUC19 plasmid vector.

2. Restriction Mapping

To generate a restriction map of the DNA inserts, plasmid DNA was digested with from 1-3 restriction enzymes and the order of restriction fragments assembled from the results. The insert DNA was then sequenced by

the dideoxynucleotide termination method (Sanger et al., Proc. Natl. Acad. Sci. USA **74**:5463 (1977)) and the resultant genomic sequence was aligned with that of the Shaker-related mouse Kv1.1 (MK1) cDNA. For Southern blotting experiments, digested DNA fragments were separated by electrophoresis on a 0.9% agarose gel and then electrotransfered to Nylon membrane (Nytran, Schleicher & Schuell, Keen, New Hampshire) using 1x Tris-acetate/EDTA transfer buffer. Electrotransfer was carried out at 4 ^{0}C for 14 hrs at 100 mA. Hybridization and washing were carried out using the same reagents and conditions described above for the library screening. Exposure of the blots was done on X-OMAT film (Kodak, Rochester, New York) at room temperature for 30 minutes.

3. DNA Sequencing

A fragment containing a majority of the coding region was cloned into pBluescript (Stratagene, La Jolla, California), and the inserts were sequenced by the dideoxynucleotide chain termination method (Sanger et al., Proc. Natl. Acad. Sci. USA **74**:5463 (1977)) using modified T7 DNA polymerase (Sequenase; US Biochemicals, Cleveland, Ohio). Plasmid-specific primers and custom designed oligonucleotide primers (purchased from Chemgenes, Needham, Massachusetts) were used for this purpose.

4. Northern Blots

For Northern blot analysis, total RNA was isolated by the guanidine thiocyanate method (Chirgwin et al., Biochemistry **18**:5294 (1979)) using the RNAgents™ total RNA isolation kit (Promega, Madison, Wisconsin). Ten nanograms of total RNA was fractionated on a 1% agarose gel after denaturation with glyoxal and dimethyl sulfoxide (McMaster and Carmichael, Proc. Natl. Acad. Sci. USA **74**:4835 (1977)) and was transferred by the

capillary method to nylon membrane (Vrati et al., Mol. Biol. Rep.(Bio-Rad Laboratories) **1(3)**:1 (1987)).

A PstI/SacI fragment from the Kv1.7-specific 3' untranslated region of the cDNA clone was radioactively labeled by the random primer method to a specific activity of $1x10^9$ cpm/microgram and used as a probe. Hybridization was performed at 55 °C in hybridization buffer consisting of 5xSSC, 10x Denhardt's and 0.1% SDS. The blot was then washed at a final stringency of 0.5xSSC and 0.1% SDS for 30 minutes at 55 °C and then exposed to X-OMAT film for 72 h at -70 °C with an intensifying screen.

5. Polymerase Chain Reaction

Total RNA isolated from mouse brain and from the hamster insulinoma cell line, HIT-TI5, was used to generate random primed cDNA by the method of Krug and Berger, Methods in Enzymology (S.L. Berger and A.R. Kimmel, Eds.) **152**:316 (1987) Academic Press, San Diego. The 40 microliter reaction mixture contained 40 units of avian myeloblastosis virus reverse transcriptase (Promega, Madison, Wisconsin), 20 units of RNasin (Promega, Madison, Wisconsin), 100 pM random hexanucleotide triphosphate (GeneAmp kit; Perkin-Elmer-Cetus, Norwalk, Connecticut). The cDNA product was then amplified for 25 cycles with an annealing temperature of 57 °C with TaqI polymerase (Promega, Madison, Wisconsin) using two oligonucleotide primers derived from the sequence of the mouse Kv1.7 genomic clone. The upstream primer 5'-TCTCCGTACTCGTCATCCTGG-3' (SEQ ID NO:20) corresponds to sequence in the S1 transmembrane segment and the downstream primer 5'-AAATGGGTGTCCACCCGGTC-3' (SEQ ID NO:21) corresponds to the 3' -> 5' complementary sequence of the carboxy terminus of the S3-S4 loop of mouse Kv1.7. The reaction mixture contained 60 mM Tris-HCl pH 8.5, 25 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 10% dimethyl sulfoxide, 0.25 microgram of each primer, 2.5 mM of each

deoxynucleotide triphosphate and 5 units of TaqI polymerase (Mullis et al., Cold Spring Harbor Symp. Quant. Biol. **51**:263 (1986)).

6. Human Chromosome Localization

Mouse genomic Kv1.7 DNA was used to isolate a human Kv1.7 cosmid clone from a human chromosome 19-enriched library (Library F) (de Jong et al., Cytogen. Cell Genet. **51**:985 (1989)), containing an approximately 4X coverage of chromosome 19 as described by Tynan et al., Nucl. Acids Res. **20**:1629 (1992) and Tynan et al., Genomics **17**:316 (1993). The probe insert fragment was isolated and labeled by random priming (Feinberg and Vogelstein, Anal. Biochem. **132**:6 (1983)) with ^{32}P-dCTP for probing. Fluorescence in situ hybridization (FISH) of cosmids to metaphase chromosomes was performed as previously described by Trask, Methods Cell Biol. **35**:3 (1990) and Trask et al., Genomics **15**:133 (1993). Two color hybridization to metaphase chromosomes was performed as described by Brandriff et al., Genomics **12**:773 (1992).

7. Expression Construct

A mouse Kv1.7 expression construct was generated by combining genomic sequences with PCR-derived cDNA sequences in the pBluescript vector, and cRNA was prepared and injected into *Xenopus* oocytes as described by Aiyar et al., 1993, Amer. J. Physiol. **265**:C1571.

8. Materials Testing

The Kv1.7 expression construct described above or related ones expressing the Kv1.7 potassium channel gene can be used to generate functional potassium channels in mammalian cell lines that do not express endogenous potassium channels by transfection of the construct into the cell line. These cell lines are then loaded with ^{86}Rb ions which permeate through potassium channels nearly as well as potassium ions. The loaded cells can then be

cultured in the presence or absence of extrinsic materials and Kv1.7 channel blockers are identified by their ability to prevent ^{86}Rb-efflux. The methods for the above experiments are all well known in the art.

9. Preparation of antibodies against the Kv1.7 potassium channels

The gene encoding the Kv1.7 potassium channel are isolated by standard recombinant DNA techniques such as described in Weir et al., Handbook of Experimental Immunology, Vol. 3 (1986) and other available documents. These genes are used as templates to prepare Kv1.7 potassium channel proteins or peptides, which are used as antigens to prepare antibodies against the Kv1.7 potassium channel. A second method for preparing antibodies against the Kv1.7 potassium channel protein is used with cells expressing large numbers of the Kv1.7 channel, isolating the cell surface proteins from these cells and using these proteins as antigens for the preparation of antibodies. The antibodies are then screened for the ability to effect Kv1.7 potassium channels electrophysiologically.

10. Drug and/or antibody testing in Type II diabetes mellitus

Materials comprising drugs or antibodies identified by assays designed to identify extrinsic materials possessing the ability to modulate the Kv1.7 potassium channel may be tested in vivo for efficacy in appropriate animal models, for example, for their ability to treat NIDDM by increasing secretion of insulin from pancreatic β-cells. The route of administration of the drugs/antibodies can be oral, parental, or via the rectum, and the drug could be administered alone as principals, or in combination with other drugs or antibodies, and at regular intervals or as a single bolus, or as a continuous infusion in standard

formations. Drugs or antibodies described supra are also tested in in vitro assays, for example, for their ability to stimulate secretion of insulin from pancreatic β-cells derived from patients or animal models of NIDDM.

11. A treatment protocol

Candidate materials identified by the assays described above are tested for safety in humans as per Federal guidelines. These candidates described supra are administered via standard formulations to diseased patients, again either orally, parenterally, rectally, alone or in combination, at regular intervals or as a single bolus, or as a continuous infusion, for modulating Kv1.7 potassium channels in pancreatic β-cells, thereby impacting on the course of the disease.

The foregoing description details specific methods that can be employed to practice the present invention. Having detailed specific methods initially used to identify extrinsic materials possessing the ability to modulate the Kv1.7 potassium channels on pancreatic β-cells, one skilled in the art will well enough know how to devise alternative reliable methods for arriving at the same basic information and for extending this information to other species including humans. Thus, however detailed the foregoing may appear in text, it should not be construed as limiting the overall scope hereof; rather, the ambit of the present invention is to be governed only by the lawful construction of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF INVENTION: A Novel Voltage-Gated Potassium Channel Gene

(iii) NUMBER OF SEQUENCES: 21

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ROBBINS, BERLINER & CARSON
(B) STREET: 201 N.Figueroa Street, 5th Floor
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: United States
(F) ZIP: 90012-2628

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/207,431
(B) FILING DATE: 04-MAR-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Berliner, Robert
(B) REGISTRATION NUMBER: 20,121
(C) REFERENCE/DOCKET NUMBER: 5555-302

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (213) 977-1001
(B) TELEFAX: (213) 977-1003
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCT GCT ACT GGC TCG GTTCTTTGTG GTGGAGA                                32
Ala Ala Thr Gly Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Ala Thr Gly Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GTCCCTTCTG CAG TTC CTC GCC CGA                                        25
               Phe Leu Ala Arg
                 1
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Phe Leu Ala Arg
  1
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GCT GCT ACT GGC TCG TTC CTC GCC CGA                                    27
Ala Ala Thr Gly Ser Phe Leu Ala Arg
  1               5
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ala Ala Thr Gly Ser Phe Leu Ala Arg
  1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GCT GCT ACT GGC TCG TTC CTC TCT CGG                                    27
Ala Ala Thr Gly Ser Phe Leu Ser Arg
  1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ala Ala Thr Gly Ser Phe Leu Ser Arg
  1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1599 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1599

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG ACT ACA AGG GAA AGC TCA AGA GAT CCA CGG AAA AGC GCC GGG TGG 48
Met Thr Thr Arg Glu Ser Ser Arg Asp Pro Arg Lys Ser Ala Gly Trp
1 5 10 15

CAG TGT TTC CAC AGG TGT GGA ACG GCA GAG GGC GCC CCT AGC CCC GCG 96
Gln Cys Phe His Arg Cys Gly Thr Ala Glu Gly Ala Pro Ser Pro Ala
20 25 30

GGG GTA ACA CCG CCC CCT CCC CCG CGC CCT GGC CGG ACT TTC CAT GCT 144
Gly Val Thr Pro Pro Pro Pro Pro Arg Pro Gly Arg Thr Phe His Ala
35 40 45

ATT TTT ACC CGC CGA CAC CGG ACA CCC GAC TGG GGT GGC TGC GGC GTC 192
Ile Phe Thr Arg Arg His Arg Thr Pro Asp Trp Gly Gly Cys Gly Val
50 55 60

GGG GCC ACA CGT CCG TTC ACC GGT CGC CCG GGC TGT GCG CGC CAT GGA 240
Gly Ala Thr Arg Pro Phe Thr Gly Arg Pro Gly Cys Ala Arg His Gly
65 70 75 80

GCC ACG GTG CCC GCC GCC CTG CGC TGC TGC GAG CGG CTG GTG CTC AAC 288
Ala Thr Val Pro Ala Ala Leu Arg Cys Cys Glu Arg Leu Val Leu Asn
85 90 95

GTG GCC GGG TTG CGC TTC GAG ACC CGC GCG CGC ACG CTC GGC CGC TTC 336
Val Ala Gly Leu Arg Phe Glu Thr Arg Ala Arg Thr Leu Gly Arg Phe
100 105 110

CCG GAC ACG CTG CTG GGG GAC CCG GTG CGC CGC AGC CGC TTC TAC GAC 384
Pro Asp Thr Leu Leu Gly Asp Pro Val Arg Arg Ser Arg Phe Tyr Asp
115 120 125

GGC GCG CGC GCC GAG TAT TTC TTC GAC CGA CAC CGG CCC AGC TTC GAT 432
Gly Ala Arg Ala Glu Tyr Phe Phe Asp Arg His Arg Pro Ser Phe Asp
130 135 140

GCG GTG CTC TAC TAC TAC CAG TCG GGC GGC CGG CTG AGA CGG CCG GCG 480
Ala Val Leu Tyr Tyr Tyr Gln Ser Gly Gly Arg Leu Arg Arg Pro Ala
145 150 155 160

CAC GTG CCC CTC GAC GTC TTC CTG GAG GAG GTG TCC TTC TAC GGG CTG 528
His Val Pro Leu Asp Val Phe Leu Glu Glu Val Ser Phe Tyr Gly Leu
165 170 175

GGG CGG CGG CTG GCG CGG CTG CGG GAG GAC GAG GGC TGC GCG GTC GCC 576
Gly Arg Arg Leu Ala Arg Leu Arg Glu Asp Glu Gly Cys Ala Val Ala
180 185 190

GAG CGG CCG CTG CCC CCG CCC TTT GCG CGT CAG CTC TGG CTG CTC TTC 624
Glu Arg Pro Leu Pro Pro Pro Phe Ala Arg Gln Leu Trp Leu Leu Phe
195 200 205

GAA TTT CCT GAG AGC TCG CAG GCT GCG CGC GTG CTC GCC GTG GTC TCC 672
Glu Phe Pro Glu Ser Ser Gln Ala Ala Arg Val Leu Ala Val Val Ser
210 215 220

GTA CTC GTC ATC CTG GTC TCC ATC GTG GTC TTT TGC CTC GAG ACA CTG 720
Val Leu Val Ile Leu Val Ser Ile Val Val Phe Cys Leu Glu Thr Leu
225 230 235 240

CCA GAC TTC CGC GAC GAC CGC GAT GAC CCG GGG CTC GCG CCG GTA GCG 768
Pro Asp Phe Arg Asp Asp Arg Asp Asp Pro Gly Leu Ala Pro Val Ala
245 250 255

GCT GCT ACT GGC TCG TTC CTC GCT CGG CTC AAT GGC TCC AGT CCC ATG 816
Ala Ala Thr Gly Ser Phe Leu Ala Arg Leu Asn Gly Ser Ser Pro Met

```
              260                 265                 270

CCA GGA GCC CCT CCC CGA CAG CCC TTC AAC GAT CCA TTC TTT GTG GTG        864
Pro Gly Ala Pro Pro Arg Gln Pro Phe Asn Asp Pro Phe Phe Val Val
        275                 280                 285

GAG ACC CTG TGT ATC TGC TGG TTC TCC TTT GAG CTG CTG GTG CAT CTG        912
Glu Thr Leu Cys Ile Cys Trp Phe Ser Phe Glu Leu Leu Val His Leu
    290                 295                 300

GTG GCC TGC CCT AGC AAA GCT GTG TTC TTC AAG AAT GTG ATG AAC CTA        960
Val Ala Cys Pro Ser Lys Ala Val Phe Phe Lys Asn Val Met Asn Leu
305                 310                 315                 320

ATT GAC TTC GTG GCC ATC CTG CCT TAC TTC GTG GCC CTG GGC ACG GAG       1008
Ile Asp Phe Val Ala Ile Leu Pro Tyr Phe Val Ala Leu Gly Thr Glu
                325                 330                 335

TTA GCC CGG CAG CGG GGT GTG GGC CAG CCG GCT ATG TCC CTG GCC ATC       1056
Leu Ala Arg Gln Arg Gly Val Gly Gln Pro Ala Met Ser Leu Ala Ile
            340                 345                 350

CTA AGG GTC ATC CGA TTG GTG CGT GTC TTC CGC ATC TTC AAG CTC TCC       1104
Leu Arg Val Ile Arg Leu Val Arg Val Phe Arg Ile Phe Lys Leu Ser
        355                 360                 365

AGG CAT TCG AAG GGT CTA CAG ATC TTG GGT CAG ACA CTG CGG GCT TCC       1152
Arg His Ser Lys Gly Leu Gln Ile Leu Gly Gln Thr Leu Arg Ala Ser
    370                 375                 380

ATG CGT GAG CTA GGT CTC CTC ATC TCC TTC CTC TTC ATT GGC GTG GTC       1200
Met Arg Glu Leu Gly Leu Leu Ile Ser Phe Leu Phe Ile Gly Val Val
385                 390                 395                 400

CTC TTT TCC AGC GCA GTC TAC TTT GCT GAA GTG GAC CGG GTG GAC ACC       1248
Leu Phe Ser Ser Ala Val Tyr Phe Ala Glu Val Asp Arg Val Asp Thr
                405                 410                 415

CAT TTC ACC AGC ATC CCG GAG TCC TTT TGG TGG GCA GTG GTC ACC ATG       1296
His Phe Thr Ser Ile Pro Glu Ser Phe Trp Trp Ala Val Val Thr Met
            420                 425                 430

ACC ACG GTT GGC TAT GGG GAC ATG GCA CCC GTC ACC GTG GGT GGC AAG       1344
Thr Thr Val Gly Tyr Gly Asp Met Ala Pro Val Thr Val Gly Gly Lys
        435                 440                 445

ATC GTG GGC TCT CTG TGT GCC ATT GCA GGT GTG CTC ACC ATC TCT CTG       1392
Ile Val Gly Ser Leu Cys Ala Ile Ala Gly Val Leu Thr Ile Ser Leu
    450                 455                 460

CCT GTG CCT GTC ATT GTC TCT AAC TTT AGC TAC TTT TAC CAC CGG GAG       1440
Pro Val Pro Val Ile Val Ser Asn Phe Ser Tyr Phe Tyr His Arg Glu
465                 470                 475                 480

ACA GAG GGC GAA GAG GCA GGG ATG TAC AGC CAT GTG GAC ACA CAG CCC       1488
Thr Glu Gly Glu Glu Ala Gly Met Tyr Ser His Val Asp Thr Gln Pro
                485                 490                 495

TGC GGT ACC CTG GAG GGC AAG GCT AAT GGG GGG CTG GTG GAC TCT GAG       1536
Cys Gly Thr Leu Glu Gly Lys Ala Asn Gly Gly Leu Val Asp Ser Glu
            500                 505                 510

GTG CCT GAA CTC CTC CCA CCA CTC TGG CCC CCT GCA GGG AAA CAC ATG       1584
Val Pro Glu Leu Leu Pro Pro Leu Trp Pro Pro Ala Gly Lys His Met
        515                 520                 525

GTG ACT GAG GTG TG                                                    1599
Val Thr Glu Val
    530
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Thr Thr Arg Glu Ser Ser Arg Asp Pro Arg Lys Ser Ala Gly Trp
 1               5                  10                  15

Gln Cys Phe His Arg Cys Gly Thr Ala Glu Gly Ala Pro Ser Pro Ala
            20                  25                  30

Gly Val Thr Pro Pro Pro Pro Pro Arg Pro Gly Arg Thr Phe His Ala
        35                  40                  45

Ile Phe Thr Arg Arg His Arg Thr Pro Asp Trp Gly Gly Cys Gly Val
    50                  55                  60

Gly Ala Thr Arg Pro Phe Thr Gly Arg Pro Gly Cys Ala Arg His Gly
65                  70                  75                  80

Ala Thr Val Pro Ala Ala Leu Arg Cys Cys Glu Arg Leu Val Leu Asn
                85                  90                  95

Val Ala Gly Leu Arg Phe Glu Thr Arg Ala Arg Thr Leu Gly Arg Phe
            100                 105                 110

Pro Asp Thr Leu Leu Gly Asp Pro Val Arg Arg Ser Arg Phe Tyr Asp
        115                 120                 125

Gly Ala Arg Ala Glu Tyr Phe Phe Asp Arg His Arg Pro Ser Phe Asp
    130                 135                 140

Ala Val Leu Tyr Tyr Tyr Gln Ser Gly Gly Arg Leu Arg Arg Pro Ala
145                 150                 155                 160

His Val Pro Leu Asp Val Phe Leu Glu Glu Val Ser Phe Tyr Gly Leu
                165                 170                 175

Gly Arg Arg Leu Ala Arg Leu Arg Glu Asp Glu Gly Cys Ala Val Ala
            180                 185                 190

Glu Arg Pro Leu Pro Pro Pro Phe Ala Arg Gln Leu Trp Leu Leu Phe
        195                 200                 205

Glu Phe Pro Glu Ser Ser Gln Ala Ala Arg Val Leu Ala Val Val Ser
    210                 215                 220

Val Leu Val Ile Leu Val Ser Ile Val Val Phe Cys Leu Glu Thr Leu
225                 230                 235                 240

Pro Asp Phe Arg Asp Asp Arg Asp Asp Pro Gly Leu Ala Pro Val Ala
                245                 250                 255

Ala Ala Thr Gly Ser Phe Leu Ala Arg Leu Asn Gly Ser Ser Pro Met
            260                 265                 270

Pro Gly Ala Pro Pro Arg Gln Pro Phe Asn Asp Pro Phe Phe Val Val
        275                 280                 285

Glu Thr Leu Cys Ile Cys Trp Phe Ser Phe Glu Leu Leu Val His Leu
    290                 295                 300

Val Ala Cys Pro Ser Lys Ala Val Phe Phe Lys Asn Val Met Asn Leu
305                 310                 315                 320

Ile Asp Phe Val Ala Ile Leu Pro Tyr Phe Val Ala Leu Gly Thr Glu
                325                 330                 335

Leu Ala Arg Gln Arg Gly Val Gly Gln Pro Ala Met Ser Leu Ala Ile
            340                 345                 350
```

```
Leu Arg Val Ile Arg Leu Val Arg Val Phe Arg Ile Phe Lys Leu Ser
    355                 360                 365

Arg His Ser Lys Gly Leu Gln Ile Leu Gly Gln Thr Leu Arg Ala Ser
370                 375                 380

Met Arg Glu Leu Gly Leu Leu Ile Ser Phe Leu Phe Ile Gly Val Val
385                 390                 395                 400

Leu Phe Ser Ser Ala Val Tyr Phe Ala Glu Val Asp Arg Val Asp Thr
                405                 410                 415

His Phe Thr Ser Ile Pro Glu Ser Phe Trp Trp Ala Val Val Thr Met
            420                 425                 430

Thr Thr Val Gly Tyr Gly Asp Met Ala Pro Val Thr Val Gly Gly Lys
        435                 440                 445

Ile Val Gly Ser Leu Cys Ala Ile Ala Gly Val Leu Thr Ile Ser Leu
    450                 455                 460

Pro Val Pro Val Ile Val Ser Asn Phe Ser Tyr Phe Tyr His Arg Glu
465                 470                 475                 480

Thr Glu Gly Glu Glu Ala Gly Met Tyr Ser His Val Asp Thr Gln Pro
                485                 490                 495

Cys Gly Thr Leu Glu Gly Lys Ala Asn Gly Gly Leu Val Asp Ser Glu
            500                 505                 510

Val Pro Glu Leu Leu Pro Pro Leu Trp Pro Pro Ala Gly Lys His Met
        515                 520                 525

Val Thr Glu Val
    530
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTATTTTTAC GNGCGGACAC CGGACTACCG 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCTGGGGCG GCGGNGG 17

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 69 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGCTCGTCCG TAGTCTCCGT GCTCCTCATC CTCGTCTCCA TCGTCGTCTT CTGCCTCGAG 60

ACGCTGCCT 69

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCCGACTCCG CTGAATGGCT CCCAGCC 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATTCTTTGTG GTGGAACCTT TGT 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATCTGCTGGT TCTCCTTTGA GCATGCTGGT GCGTCTGGCG GCGTGTCCAA GCAAAGCTGT 60

ATTTTTCAAG AATGTGATGA ACCTTATTGA CTT 93

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTGGCCATCC TGCCTTACTT TGTGGCCCTG GGCACAGAGT TAGCC 45

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCAGCGGGG CGTGGGCCAG CCAGCTATGT CCCTGGCCAT CCTGAGGAGT CATCNGATTG 60

GTGCGTAGTC TTCCGCATCT TCAAGCTNTC CNGGCANTCN AAGGGCNTGC AAATCTTGGG 120

CCAGGACGCT TCGGGCCTCC ATGCGTGAAG CTGGGCCTCC TCATCTTTTT CCTCTTCATC 180

GGTGTGGTCC TCTTTT 196

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 271 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTTCCCTGCC AGTGCCCGTC ATTGTCTCCA ATTTCAGCTA CTTTTATCAC CGGGAGACAG 60

AGGGCGAAGA GGCTGGGATG TTCAGCCATG TGGACATGCA GCCTTGTGGC CCACTGGANG 120

GNNCANGNCN ANNCCAATGG GGGGCTGGTG GACGGGGAGG TACCTGAGCT ACCACCTCCA 180

CTCTGGGCAC CCCCAGGGAA ACACCTGGTC ACCGAAGTGT GAGGAACAGT TGAGGTCTGC 240

AGGAATTCGA TATCAAGCTT ATCGATACCG T 271

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TCTCCGTACT CGTCATCCTG G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAATGGGTGT CCACCCGGTC 20

WHAT IS CLAIMED IS:

1. An isolated DNA molecule having a sequence (SEQ ID NO:9) as set forth in Figure 3.

2. A method of using the DNA molecule of Claim 1 as a template for expression thereof.

3. The product of the method according to Claim 2.

4. The product according to Claim 3 wherein said product is the mouse Kv1.7 potassium channel.

5. An assay for screening and identifying extrinsic materials having a modulating effect on Kv1.7 potassium channels comprising the steps of:

a) providing a culture of cells expressing the Kv1.7 potassium channel,

b) contacting said culture of cells with one or more of a battery of test materials that can potentially modulate the Kv1.7 potassium channel thereof,

c) monitoring the effect of said test materials on the Kv1.7 potassium channel, and

d) selecting a candidate or candidates from the battery of test materials capable of modulating the Kv1.7 potassium channel.

6. An assay according to Claim 5 wherein the monitoring of step c) is conducted by measuring the rate of ^{86}Rb efflux from a ^{86}Rb loaded cell expressing the Kv1.7 potassium channel.

7. An assay according to Claim 6 wherein the selecting of step d) is based upon a test extrinsic material inducing little or less than normal ^{86}Rb efflux from said cell.

FIG. 1A


```
mKv1.7genomic GCTGCTACTGGCTCG GTTCTTTGTGGTGGAGA..[1.9kb]..GTCCCTTCTGCAG TTCCTCGCCCGA
              AlaAlaThrGlySer                                           PheLeuAlaArg
   mKv1.7cDNA GCTGCTACTGGCTCG                                           TTCCTCGCCCGA
  haKv1.7cDNA GCTGCTACTGGCTCG                                           TTCCTCTCTCGG
```

FIG. 1B

```
MTTRESSRDPRKSAGWQCFHRCGTAEGAPSPAGVTPPPPPRPGRTFHAIFTRRHRTPDWGGCGVGATRPFTGRPGCARHG 80
                                                                 TY-K
ATVPAALRCCERLVLNVAGLRFETRARTLGRFPDTLLGDPVRRSRFYDGARAEYFFDRHRPSFDAVLYYYQSGGRLRRPA 160
                                                                    S1
HVPLDVFLEEVSFYGLGRRLARLREDEGCAVAERPLPPPFARQLWLLFEFPESSQAARVLAVVSVLVILVSIVVFCLETL 240
                     *                                    S2
PDFRDDRDDPGLAPVAAATGSFLARLNGSSPMPGAPPRQPFNDPFFVVETLCICWFSFELLVRLVACPSKAVFFKNVMNL 320
                                                                   PKC
       S3                             S4                                   S5
IDFVAILPYFVALGTELARQRGVGQPAMSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLRASMRELGLLISFLFIGVV 400
                                   P                        S6
LFSSAVYFAEVDRVDTHFTSIPESFWWAVVTMTTVGYGDMAPVTVGGKIVGSLCAIAGVLTISLPVPVIVSNFSYFYHRE 480

TEGEEAGMYSHVDTQPCGTLEGKANGGLVDSEVPELLPPLWPPAGKHMVTEV 532
```

FIG. 2

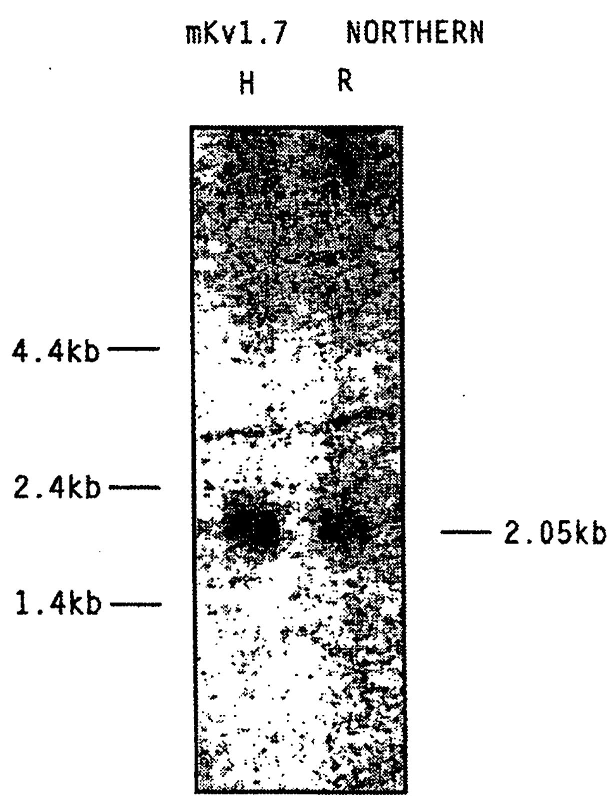


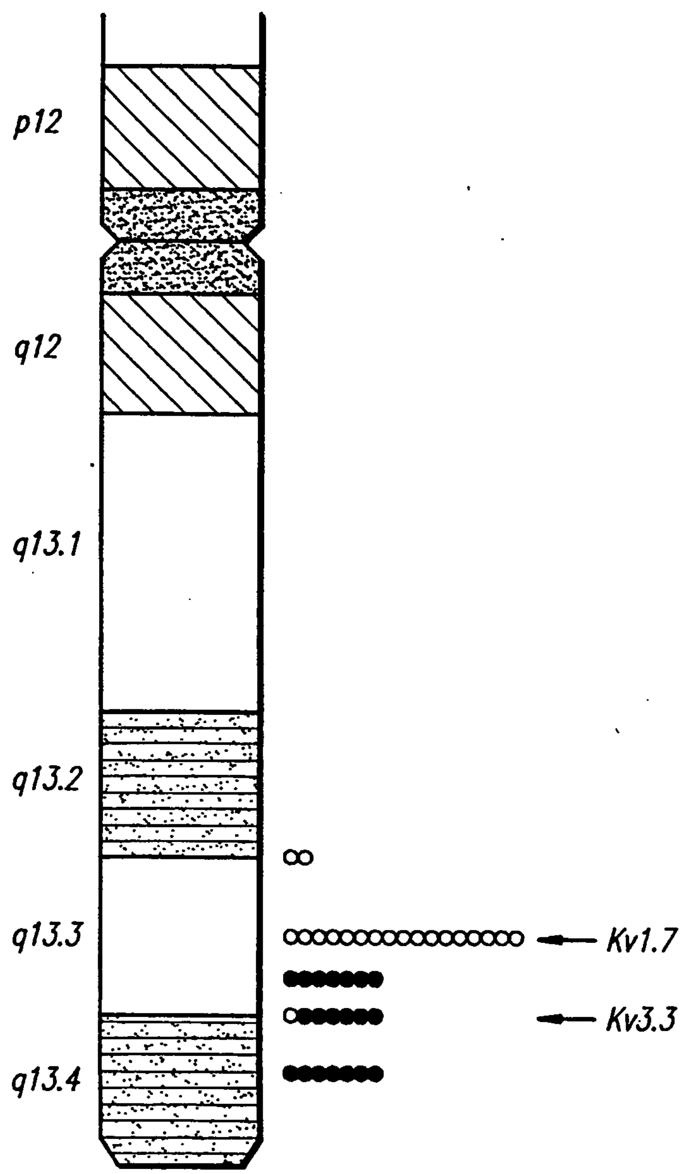
FIG. 3A

```
ATGACTACAAGGGAAAGCTCAAGAGATCCACGGAAAAGCGCCGGGTGGCAGTGTTTCCAC  60

AGGTGTGGAACGGCAGAGGGCGCCCCTAGCCCCGCGGGGGTAACACCGCCCCCTCCCCCG 120
                     ---- ----                 ----  -G--GG-

CGCCCTGGCCGGACTTTCCATGCTATTTTTACCCGCCGACACCGGACACCCGACTGGGGT 180
                      ----------GN--G----------TA---1

GGCTGCGGCGTCGGGGCCACACGTCCGTTCACCGGTCGCCCGGGCTGTGCGCGCCATGGA 240
-----G----G---N-G2

GCCACGGTGCCCGCCGCCCTGCGCTGCTGCGAGCGGCTGGTGCTCAACGTGGCCGGGTTG 300

CGCTTCGAGACCCGCGCGCGCACGCTCGGCCGCTTCCCGGACACGCTGCTGGGGGACCCG 360

GTGCGCCGCAGCCGCTTCTACGACGGCGCGCGCGCCGAGTATTTCTTCGACCGACACCGG 420

CCCAGCTTCGATGCGGTGCTCTACTACTACCAGTCGGGCGGCCGGCTGAGACGGCCGGCG 480

CACGTGCCCCTCGACGTCTTCCTGGAGGAGGTGTCCTTCTACGGGCTGGGGCGGCGGCTG 540

GCGCGGCTGCGGGAGGACGAGGGCTGCGCGGTCGCCGAGCGGCCGCTGCCCCCGCCCTTT 600

GCGCGTCAGCTCTGGCTGCTCTTCGAATTTCCTGAGAGCTCGCAGGCTGCGCGCGTGCTC 660
                                                      3-----

GCC GTGGTCTCCGTACTCGTCATCCTGGTCTCCATCGTGGTCTTTTGCCTCGAGACACTG 720
-T-C--A--------G---C-------C-----------C-----C-----------G---

CCAGACTTCCGCGACGACCGCGATGACCCGGGGCTCGCGCCGGTAGCGGCTGCTACTGGC 780
--T -----

TCGTTCCTCGCTCGGCTCAATGGCTCC AGTCCCATGCCAGGAGCCCCTCCCCGACAGCCC 840
     4--CGA---C---G---------C--C- AN-

TTCAACGATCCATTCTTTGTGGTGGAGACCCTGTGTATCTGCTGGTTCTCCTTTGAGC TG 900
           5---------------AC-TT---  6---------------------A--
```

FIG. 3B

```
CTGGTGCATCTGGTGGCCTGCCCTAGCAAAGCTGTGTTCTTCAAGAATGTGATGAACCTA  960
-------G-----C---G--T--A-----------A--T--------------------T

ATTGACTTCGTGGCCATCCTGCCTTACTTCGTGGCCCTGGGCACGGAGTTAGCCCGGCAG 1020
-------- -------------------T--------------A--------- -T---
         7                                             8
CGGGGTGTGGGCCAGCCGGCTATGTCCCTGGCCATCCTAAGG GTCATCCGATTGGTGCGT1080
-----C-----------A--------------------G---A------N-----------A

GTCTTCCGCATCTTCAAGCTCTCCAGGCATTCGAAGGGTCTACAGATCTTGGGTCAG ACA1140
--------------------N---N----N--N-----CN-G--A--------C---G--G

CTGCGGGCTTCCATGCGTGA GCTAGGTCTCCTCATCTCCTTCCTCTTCATTGGCGTGGTC1200
--T-----C-----------A---G--C----------TT-----------C--T------

CTCTTTTCCAGCGCAGTCTACTTTGCTGAAGTGGACCGGGTGGACACCCATTTCACCAGC 1260
-------

ATCCCGGAGTCCTTTTGGTGGGCAGTGGTCACCATGACCACGGTTGGCTATGGGGACATG 1320

GCACCCGTCACCGTGGGTGGCAAGATCGTGGGCTCTCTGTGTGCCATTGCAGGTGTGCTC 1380

ACCATCTCTCTGCCTGTGCCTGTCATTGTCTCTAACTTTAGCTACTTTTACCACCGGGAG 1440
    -T--C-----A-----C-----------C--T--C-----------T---------
    9
ACAGAGGGCGAAGAGGCAGGGATGTACAGCCATGTGGACACACAGCCCTGCGGTACCCTG 1500
-----------------T-------T------------TG-------T--T--CC-A---

GAGGG  CAAGGCTAAT    GGGGGGCTGGTGGACTCTGAGGTGCCTGAACTCCTCCCAC1555
--N--NN-N-N-N-NNCCAAT---------------GGG-----A-----G--A-CA--T-

CACTCTGGCCCCCTGCAGGGAAACACATGGTGACTGAGGTGTGA  (END)         1599
--------G-A--CC-----------C----C--C--A------GGAACAGTTGAGGTCTG

CAGGAATTCGATATCAAGCTTATCGATACCGT
```

FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02221

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/12, 5/10; C07K 14/705; G01N 33/50, 33/60
US CL :536/23.5; 435/69.1, 7.2, 29; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.5; 435/69.1, 7.2, 29; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	*European Biophysics Journal,* Volume 23, Number 5, issued December 1994, A. Bertoli *et al.,* "Activation and deactivation properties of rat brain K^+ channels of the *Shaker*-related subfamily", pages 379-384.	1-7
A	*FEBS Letters,* Volume 263, Number 1, issued 09 April 1990, C. Betsholtz *et al.,* "Expression of voltage-gated K^+ channels in insulin-producing cells: Analysis by polymerase chain reaction", pages 121-126.	1-7
A	*Proceedings of the National Academy of Sciences USA,* Volume 88, Number 1, issued January 1991, L. H. Philipson *et al.,* "Sequence and functional expression in *Xenopus* oocytes of a human insulinoma and islet potassium channel", pages 53-57.	1-7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
10 MAY 1995

Date of mailing of the international search report
18 MAY 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
DAVID L. FITZGERALD
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02221

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Trends in Pharmacological Sciences, Volume 14, Number 12, issued December 1993, K. G. Chandy et al., "Nomenclature for mammalian potassium channel genes", page 434.	1-7

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Sequence databases: GenBank/EMBL/DDBJ, GeneSeq, SwissProt, PIR
Keyword databases: Biosis, SciSearch, Embase, Medline, CAS, EPO online, Derwent WPI, USPTO-APS
search terms: shaker; intron?; potassium/K channel; voltage dependent; diabetes, insulin, iddm, pancreatic beta, rinm5f